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AUTHORITY

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VARYING BEHAVIOR OF PASTEURELLA-PHAGES

(Received on 15 May 1963)

[Following is a translation of an article by W. Knapp, Hygiene-Institute, University of Tubingen, in the German-language periodical Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene (Journal for Bacteriology, Parasitology, Infectious Diseases and Hygiene), Vol 190, 1963, pages 39-46.]

It is known that Past. pestis-phages and Past. pseudotuberculosis-phages show differences in their lytic behavior toward homologous and heterologous bacteria strains, which could disappear under certain conditions after adaptation of the individual phage strains to a heterologous bacteria strain (references 4, 5, 6, 7 and 11). It was the aim of this work to determine whether the phage strains described as Past. pestis-phages or Past. pseudotuberculosis-phages exhibit differences in their behavior toward various effects of their surroundings or immunsera.

The behavior of the individual phage strains with respect to the following was examined:

1. changing temperatures and times of action
2. chemical substances (phenol, chloroform, merthiolate and formalin), normal serum and changing pH as well as
3. in a crosswise neutralization test toward various antiphage sera.

Experimental Material

A. Pasteurella-phages: PST-phage; Past. pseudotuberculosis-phage strains, supplied by Prof. Dr. Girard, Pasteur Instituto, Paris.

Y-phage; "Yersin" pestis-phage strains, supplied by Prof. Dr. Girard, Pasteur Institute, Paris.

PTB-phage; Advier pestis-phage strain, adapted by Gunnison and co-workers to Past. pseudotuberculosis; supplied by Prof. Dr. Gunnison, San Francisco; School of Medicine, University of California.

R-phage; described as Past. pseudotuberculosis-phage by Koltjarowa (1956, 8) and supplied by Prof. Dr. V. N. Ter-Vartanov, Director of the Anti-Pest-Institute of the Caucasus and Trans-caucasus, Stavropol.

The designation R-phage ("Russian phage") was chosen by us[1].

B. Salmonella-phages; salmonella O_1-phage; supplied by Dr. Brandis, Hygiene-Institute, Gottingen[2].

C. Bacteria strains and nutrients: as described in a previous paper (Knapp, 1962).

Experimental Method

The separate enrichment of Pasteurella-phage strains over Past. pestis strains as well as pseudotuberculosis strains was carried out in liquid or solid nutrient media according to the known technique (References 1 and 2). The titers of the phage suspensions before and after their thermal or chemical treatment were determined exclusively according to the method of overlay. In all initial mixtures, 2.5 milliliters soft agar containing 1 drop of a 24-hour proteose inclined-agar culture of the indicator bacteria was treated with five milliliters proteose solution and 1.0 milliliter

1/ The Past. pseudotuberculosis-phage strains described by Plankina and co-worker (1961; 9) have not been available as yet.

2/ We are grateful to Professors H. Brandis, S. Girard, I. Gunnison, K. F. Meyer and V. N. Ter-Vartanov for letting us have the various strains.

phage suspension. The inoculation of the mixture was carried out on pre-dried proteose-agar plates. We kept the pretreated and untreated phage suspensions in a refrigerator at 2° to 4° C.

For the preparation of the anti-phage immunsera, increasing amounts of phage were injected into rabbits at first intramuscularly and subsequently intravenously. Two renewal injections followed in a period of two days after an injection-free period of approximately two months. We did not consider a saturation of all sera after preliminary experiments with unsaturated immunsera, some of them containing an agglutinate titer up to 1:160 serum dilution with respect to the bacteria strain used for the enrichment of the phages, yielding the same results in the neutralization tests as saturated immunsera. (For further details see Table 3).

Results

1. Temperature sensitivity

Table 1 shows the behavior of the phage strains at various temperatures and times of actions.

As may be seen from Table 1, the Y-, PTB-, and R-phage strains are more temperature sensitive than PST-phage and salmonella O_1-phage which had been included in the study for comparison. While an inactivation of the Y-, PTB- and R-phages at 60° C occurs already within 5 to 10 minutes, this inactivation took place during approximately the same time period, but only at 70° C, in the case of PST-phage.

The temperature sensitivity of the phage strains did not change when they were enriched over a heterologous strain--for instance, when PST-phage was enriched over the Past. pestis strain TWJ, the pest phage Y over Past. pseudotuberculosis strain No 2I and the pest phage PTB adapted on Past. pseudotuberculosis over Past. pestis-strain TWJ. The PTB- and R- phages show a temperature sensitivity corresponding to Y-phage; this temperature sensitivity remained the same, although both phage strains had been enriched over Past. pestis of Past. pseudotuberculosis.

Our experimental results have been summarized in Table 1.

Table 1

Temperature Sensitivity of the Phage Strains

Temperature °C	Time of action* min.	PST				
56	5	$7 \cdot 10^4$	cfl**	cfl	$2 \cdot 10^4$	cfl
	30	$7 \cdot 10^4$	$2 \cdot 10^4$	$2 \cdot 10^3$	$3 \cdot 10^3$	cfl
	60	$6 \cdot 10^4$	$4 \cdot 10^4$	$6 \cdot 10^4$	$2 \cdot 10^4$	cfl
60	5	$3 \cdot 10^4$	$9 \cdot 10^4$	—***	10^4	$5 \cdot 10^4$
	30	$2 \cdot 10^4$	—	—	—	$3 \cdot 10^4$
	60	$9 \cdot 10^3$	—	—	—	$4 \cdot 10^4$
65	5	$7 \cdot 10^3$		—		$2 \cdot 10^3$
	30	$2 \cdot 10^3$				$2 \cdot 10^3$
	60	$3 \cdot 10^2$	—		—	10^3
70	5	—		—	—	10^3
	15	—	—	—	—	10^3
	30	—	—	—	—	$2 \cdot 10^2$
75	5	—	—	—	—	—
	15	—	—	—	—	—

*Titer determinations were carried out after 5, 10, 15, 20, 30, 45 and 60 minutes. For reasons of simplicity only the values after 5, 30, and 60 minutes were given from 56° to 65° C and only the 5, 15 and 30 or 5 and 15 minute values were given at 70° and 75° C.

**cfl = confluent lysis, plaques uncountable.

***___ = no plaques detectable

2. Sensitivity towards chemical actions

The inactivating effect of phenol and formalin in 0.25 and 0.5 percent concentration, of chloroform in 1 and 10 percent concentration, of merthiolage in 0.01 percent concentration, of undiluted and 1:10 diluted human normal serum and of solutions with pH 3-9 was studied, usually after a time of action of 1, 3, 6, 24 and 48 hours, 2 and 4 weeks or 2 or 4 months (exceptions see footnote Table 2).

The experimental results for human normal serum will be reported at another place.

For the preparation of, for instance, the phage suspension in a 0.25 percent phenol solution, 1 milliliter of a 2.5 percent phenol solution and 1 milliliter of a concentrated phage suspension were added to 8 milliliters of proteose solution. After the various times of action had elapsed, the amount of phenol phage suspension necessary for the experiment was further diluted until the titer to be used was obtained. The phenol was hereby diluted to such an extent that an effect inhibiting the growth of the test nucleus did not occur. The remaining test solutions were treated correspondingly.

Table 2

Sensitivity of the Phage Strains Toward Various Chemical Actions

Test Compound	Concentration % p_H-Levt	No or incomplete inactivation* of the phages after hours(hr), days(d), or months(mo); longest observation period — Phage Strains PST	Y**	R	O_1 (Titer: see Table 1)
Phenol	0.25	No inactivation of the phages in 4 months			
	0.50				
Chloroform	1.0	No inactivation of the phages in 4 months			
	10.0				
Merthiolate	0.01	No inactivation of the phages in 4 months			
Formalin	0.25	1-3 hr	1-3 hr	1 hr	1-3 hr
	0.50	1 hr	1 hr	1 hr	1 hr
pH***	3.0	6 hrs	6 hrs	6 hrs	6 hrs
	4.0	none/1 mo	6 hrs	6 hrs	none/1 mo
	5.0	none/1 mo	none (Titer Decrease)	none	none/1 mo
	6.0-8.0	no inactivations of the phages in 1 mo			
	9.0	none	none (Titer Decrease)	none	none

*Inactivation no detection of plaques. A titer decrease as

a sign of partial inactivation of the phages is not considered in the Table with few exceptions.
**Same behavior of PTB-phage.
***Test for inactivation after 6, 24, and 48 hours, as well as 1 month.

The results summarized in Table 2 show that:

a) in contrast with formalin, phenol does not possess any inactivating effect on the tested phage strains in the concentrations and times of action studied;
b) these phage strains behave equally towards formalin, chloroform and merthiolate and besides they behave like other phages known from the literature (1, 10, 11);
c) the Y-, PTB- and R- phage strains were more sensitive than PST-phage and O_1-phage toward pH 4.0-5.0.

3. Behavior of the phage strains toward phage immunsera in the neutralization test

The neutralizing effect of the following rabbit immunsera was tested:

Serum No 2285: PST-phage enriched over Past. pseudotuberculosis strain 2^I.

Serum No 3046: Y-phage enriched over Past. pestis, strain A 1122.

Serum No 900 : R-phage enriched over Past. pseudotuberculosis, strain 2^I.

Serum No 1376: R-phage enriched over Past. pestis, strain TWJ.

Each serum was tested at 1:40 to 1:1280 dilution for each neutralizing effect on PST-, Y- and R- phages. In each case, the phage strains had been enriched over Past. pestis or Past. pseudo-tuberculosis. The initial mixture for the experiments consisted of 1.5 milliliter of the diluted serum with 1.5 milliliter of the phage suspension (see Table 3 for titer). After an action period of one hour, 1.0 milliliter of the serum-phage mixture was added to 2.5 milliliters sof.

agar containing 1 drop indicator culture. Pre-dried proteose-agar plates were coated with the mixture. The Past. pestis-strain TWJ used for enrichment and Past pseudotuberculosis strain 2 were served as indicator cultures. The results of these experimental series are shown in Table 3.

Table 3

Behavior of Phage Strains Toward Immunsera (neutralization test)

Phage	[illegible] Titer of the phage suspension	Indicator strain	2285	3018	1376	[illegible]
			[illegible]	[illegible]	[illegible] K rn TWJ	[illegible]
PSF	P. pathc. (21) Titer [illegible]	21	160*	L**	L	L
	P. pestis (TWJ) Titer 6 · [illegible]	TWJ	320	L	L	L
Y	P. pathc. (21) Titer [illegible]	21	L	1280	640	320
	P. pestis (TWJ)*** Titer: 1 · [illegible]	TWJ	L	640	80	320
PTB	P. pathc. (21) Titer [illegible]	21	L	80	80	80
	P. pestis (TWJ) Titer: 3 · [illegible]	TWJ	L	40	40	160
R	P. pathc. (21) Titer: 2 · [illegible]	21	L	160	320	640
	P. pestis (TWJ) Titer: 1 · [illegible]	TWJ	L	80	160	640

NOT REPRODUCIBLE

* Numbers = serum dilution still inhibiting phage effect (for instance 1:160 etc.).

** L = phage effect (lysis or numerous plaques) not inhibited by immunserum (dilution 1:40), comparison with serum-free control.

*** TWJ = use of Past. pestis-strain TWJ instead of strain A1122, because the latter pest strain exhibited degeneration phenomena.

The experimental tests obtained in the experiments show:

a) the Y-, PTB- and R- phage strains behave in the neutralization test largely identical. Their lytic effect was inhibited by anti-Y-phage serum (No 3046) and the two anti-R-phage sera (No 1376 and No 900), but not by anti-PST-phage serum (No 2285). On the other hand, serum No 2285 inhibited only the PST-phage strain; but the lytic effect of the latter was not affected by the anti-Y- and anti-R-phage sera (No 3046, 1376 and 900).

b) The enrichment of the pest-and pseudotuberculosis-phage strains over a heterologous Pasteurella-strain and the testing of these phage suspensions in the neutralization test toward the bacteria strain used for the enrichment as indicator strain does not permit detection of change of their behavior toward immunsera prepared with the homologous or the heterologous phage strain.

The kinetics of the phage inactivation by a certain dilution of the various anti-phage sera was tested in further experiments. Also in these experiments, which will be published on another occasion, a similar behavior of the Y-, PTB-, and R- phages was found. For instance, anti-PST-phage serum No 2285, when diluted to 1:200, led within 45 minutes to a decrease in the PST-phage titer (indicator strain 2^2) from $5 \cdot 10^8$ to $7 \cdot 10^4$ and after 60 minutes no plaques occurred. The titer of Y-, PTB- and R- phages, on the other hand, was not considerably affected.

These studies of the inactivation kinetics of various Pasteurella-phages by immunsera, which had been prepared with homologous or heterologous phage strains, respectively, shall also provide an answer to the question whether the phage strains do not exhibit, on account of a change in their serological properties, a different inactivation-property by the homologous or heterologous immunsera with simultaneous testing against a homologous or heterologous indicator strain, after enrichment of the phage strains via homologous strains or adaptation to and enrichment via the heterologous Pasteurella strain.

These results, as well as those summarized under 1. and 2., which show a behavior different from PST-phage,

but [illegible] Y-, PTB- and R- phage, lead to the question whether the R- phage described as Past. pseudotuberculosis phage is not a pest phage adapted to Past. pseudotuberculosis.

The answer to this question is based on the study of further Past. pseudotuberculosis-phage strains which, certainly, just like the PST-phage strain, have now come into contact with Past. pestis, but which had not been available.

According to Kotljarowa (1956; S) the"R-phage" described as pseudotuberculosis-phage lysated 41 of 42 tested Past. pseudotuberculosis strains which were all better lysated in R-form than in S-form. A strain, only present in S-form, was not lysated. Furthermore, all 15 tested pest strains were lysated, but none of the 32 cultures of genus of the species Salmonella, Shigella and Escherichia were lysated. Its inactivation was reported to occur at 56° or 65° C within 90 or 30 minutes, respectively.

Summary

The behavior of two strains of Past. pseudotuberculosis-phages and two others described as Past. pestis-phages one of which (PTB-phage) was adapted to Past. pseudotuberculosis was studied in regard to thermic (56°-75° C) and chemical factors (phenol, chloroform, merthiolate, formalin, pH 3-9). Also, cross-wise neutralisation- and inactivation-tests with phage-immunsera were carried out. The results ~~of these studies are listed and~~ discussed. They show that the Past. pseudotuberculosis-phage called PST-phage is more resistant to temperatures of 56°-65° C and to solutions with pH 4.0 to 5.0 than the three Y-, PTB- and R- strains described as Past. pestis- or Past. pseudotuberculosis-phages and which reacted alike. The difference in the behavior of PST- and Y-phages on one hand and the uniform behavior of Y-, PTB- and R- phages on the other hand suggest that the R-phages also represent a primary strain of Past. pestis-phages which is adapted to Past. pseudotuberculosis. Further experiments have to be done to show whether this varying behavior is common to a larger number of Past. pestis and Past. pseudotuberculosis-phages.

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For further detailed literature references see references 1, 2, 4, 6, 7 and 11.

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